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## **Tissue engineering: A new approach in cardiovascular surgery; Seeding of human fibroblasts followed by human endothelial cells on resorbable mesh<sup>1</sup>**

Zünd, Gregor ; Hoerstrup, Simon P ; Schoeberlein, Andreina ; Lachat, Mario ; Uhlschmid, Georg ; Vogt, Paul R ; Turina, Marko

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# Tissue engineering: A new approach in cardiovascular surgery; Seeding of human fibroblasts followed by human endothelial cells on resorbable mesh<sup>1</sup>

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## Abstract

**Objective:** In tissue engineering the material properties of synthetic compounds are chosen to enable delivery of dissociated cells onto a scaffold in a manner that will result in in vitro formation of a new functional tissue. The seeding of human fibroblasts followed by human endothelial cells on resorbable mesh is a precondition of a successful creation of human tissues such as vessels or cardiac valves. **Methods:** Polymeric scaffolds ( $n = 18$ ) composed of polyglycolic acid (PGA) with a fiber diameter of 12–15  $\mu\text{m}$  and a polymer density of 70 mg/ml were used as square sheets of  $1 \times 1 \times 0.3$  cm. Fibroblasts (passage 7) harvested from human foreskin were seeded ( $3.4 \times 10^6$ ) and cultured over a 3 week period on a PGA-mesh, followed by seeding of endothelial cells (passage 5,  $2.8 \times 10^6$ ) harvested from human ascending aorta. Thereafter the new tissue was stained for HE, van Gieson, Trichrom–Masson, Factor VIII and CD 34 and proved by scanning electron microscopy. **Results:** Microscopic examination of the seeded mesh demonstrated that the human fibroblasts were attached to the polymeric fibers and had begun to spread out and divide. The scanning electron microscopic examination demonstrated a homogeneous scaffold resembling a solid sheet of tissue. The seeded endothelial cells formed a monolayer on the fibroblasts and no endothelial cell invasion or new formation of capillaris could be detected. **Conclusions:** These results are a first step to demonstrate that seeding of human fibroblasts and endothelial cells on PGA-mesh might be a feasible model to construct human tissues such as vessels or cardiac valves. © 1998 Elsevier Science B.V. All rights reserved.

**Keywords:** Tissue engineering; Synthetic biodegradable matrix; Endothelial cells

## 1. Introduction

Transplantation of endothelial cells onto the blood flow surface of a small-diameter vascular conduit, homografts, or valve prosthesis to partially reproduce the antithrombogenic lining of a naturally occurring intima is a logical concept. In our experiences, endothelial cell transplantation has failed to make a significant transi-

tion from the animal laboratory to the clinical arena [1]. We believe nowadays that human endothelial cells need a living compound for normal growth and survival in the human blood stream.

Tissue engineering is a multidisciplinary science that utilizes basic principles from engineering and life sciences to construct tissues from their cellular components [2]. For the creation of new functional tissue three general strategies have been adopted [3]. First, isolated cells or cell substitutes can be used; this technique allows the replacement of only those cells that supply the needed function. Second, tissue-inducing substances such as growth factors can be used to stimulate the

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growth of the desired cell population. Third, cells can be cultured on or within specific matrices.

These days, our laboratory has focused on the creation of new functional tissue substitutes utilizing human cells attached to synthetic, biodegradable polymer scaffolds. This approach involves isolating cells from a donor and seeding these cells onto a polymeric scaffold, where they attach and grow and finally form a tissue-like structure. The cell-polymer construct is configured to serve as a template to guide this development. Preliminary animal studies have demonstrated the feasibility of this strategy with the successful formation of a tissue engineered valve leaflet and a successful implantation of this leaflet with good functional results [4–6]. The ability to isolate cells types is an important component to any tissue engineering project. Furthermore, the seeding of human fibroblast followed by human endothelial cells on resorbable mesh is a precondition of the creation of any tissue in the cardio-vascular field.

## 2. Material and methods

### 2.1. Scaffold

Polymeric non-woven scaffold, composed of polyglycolic acid (PGA) with a thickness of 3 mm, a fiber diameter of 12–15  $\mu\text{m}$  and a polymer density of 70 mg/ml (Albany International Research, Mansfield, MA, USA; a kind gift from Dr. J. Vacanti, Children's Hospital, Harvard Medical School, Boston, MA, USA) were used as square sheets of  $0.3 \times 1 \times 1$  cm. The PGA-polymer by hydrolysis has an in vitro resorption rate of 4–6 weeks.

### 2.2. Human fibroblasts

Human foreskins were obtained from phimosiopérations performed at Children's Hospital, University Zurich. The harvested foreskins were serially washed with phosphate buffered saline (Gibco BRL-Life Technologie, Grand Island, NY) supplemented with 1% antibiotic/antimycotic solution saline (Gibco BRL-Life Technologie, Grand Island, NY). Under a laminar flood hood (Forma Scientific, Marietta, OH), the tissue was minced into 1–2 mm pieces and evenly distributed in  $15 \times 60$  mm tissue culture dishes (Becton Dickinson, Lincoln Park, NJ). Medium was gently added to the tissue culture dishes. Medium consisted of Dulbecco's modified Eagle's medium (Gibco BRL-Life Technologies) supplemented with 10% fetal bovine serum (Gibco BRL-Life Technologies) and 1% antibiotic/antimycotic solution (Gibco BRL-Life Technologies). The explanted tissue was placed in a humidified incubator (Forma scientific) at

37°C with 5%  $\text{CO}_2$  for 2–3 weeks. During this period, the cells were migrated off the explants. Thereafter cells were serially passaged to obtain sufficient numbers of cells for cell seeding.

### 2.3. Human aortic endothelial cells

Human aortic endothelial cells were obtained from the ascending aorta of the explanted sick hearts of heart transplant patients. The harvested piece of tissue of ascending aorta was serially washed with phosphate buffered saline (Gibco BRL-Life Technologies, Grand Island, NY) supplemented with 1% antibiotic/antimycotic solution (Gibco BRL-Life Technologies). Under a laminar flood hood (Forma Scientific, Marietta, OH), the intima surface was covered with Collagenase Typ I (75 U/ml in PBS pH 7.4, Worthington) and incubated for 30 min at room temperature. Cells were detached using a rubber cell scraper and cultured in 75  $\text{cm}^2$  culture dishes (Becton Dickinson Lincoln Park, NJ). Medium 199 with EBS (Amimed, Allschwil, Switzerland) supplemented with 10% fetal bovine serum, 16 U/ml Heparin, 20  $\mu\text{g/ml}$  Endothelial cell growth supplement (Becton, Dickinson) and 1% antibiotic. The explanted cells were placed in a humidified incubator at 37°C with 5%  $\text{CO}_2$  for 2–3 weeks. Cells were serially passaged to obtain sufficient numbers of cells for cell seeding. The cells were then labeled with an acetylated low density lipoprotein (Ac-Dil-LDL) probe (Biomedical Technologies, Stoughton, MA) which selectively fluorescently tags endothelial cells through their LDL receptor. These cells populations were then checked for their purity by fluorescent microscopy.

### 2.4. Seeding

The PGA polymers ( $n = 18$ ,  $1 \times 1 \times 0.3$  cm) were seeded with human fibroblasts (passage 7). There were 8–10 seeding procedures every 90 min. Each seeding procedure contained  $3.4 \times 10^6$  human fibroblasts. The seeded fibroblasts were cultured over a 21 days period. Media consisted of Dulbecco's modified Eagle's medium (Gibco BRL-Life Technologies) supplemented with 10% fetal bovine serum (Sigma, St. Louis, MO) and 1% antibiotic solution (Irvine Scientific, Santa Clara, CA). The medium was changed every 4 days. Thereafter this tissue-like structure was seeded with the pure human aortic endothelial cell culture (passage 5,  $2.8 \times 10^6$ ). The constructs were then fixed with 0.4% paraformaldehyde and stained by conventional histological methods (Hemalaun Eosin, Van Gieson, Trichrom-Masson) and examined by electron microscopy.

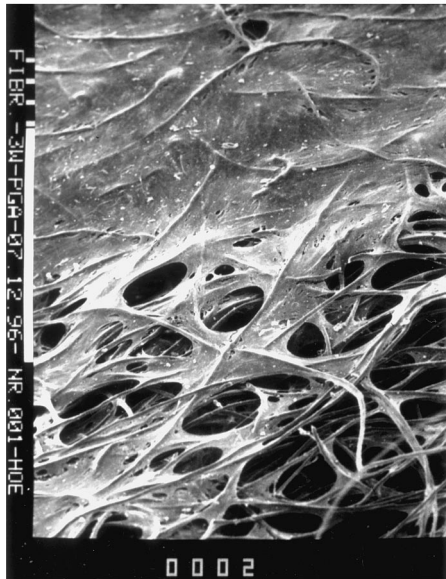


Fig. 1. Formation of fibroblast-polymer structure. Scanning electron microscopy demonstrates the formation of a continuous fibroblast tissue.

### 3. Results

#### 3.1. Conventional microscopy

Conventional microscopy examination demonstrated that the seeded human fibroblasts were attached to the polymeric fibers and had begun to spread out and divide (Figs. 1 and 2). Furthermore the seeded cells were forming cell bridges and a homogeneous tissue.

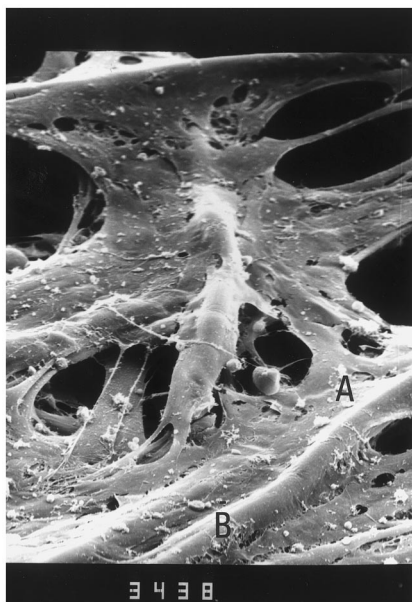


Fig. 2. Continuous formation of fibroblasts. Scanning electron microscopy shows a continuous distribution of fibroblasts throughout the 'polymeric architecture' and attachment of the fibroblasts (A) to the polymer fibers (B).

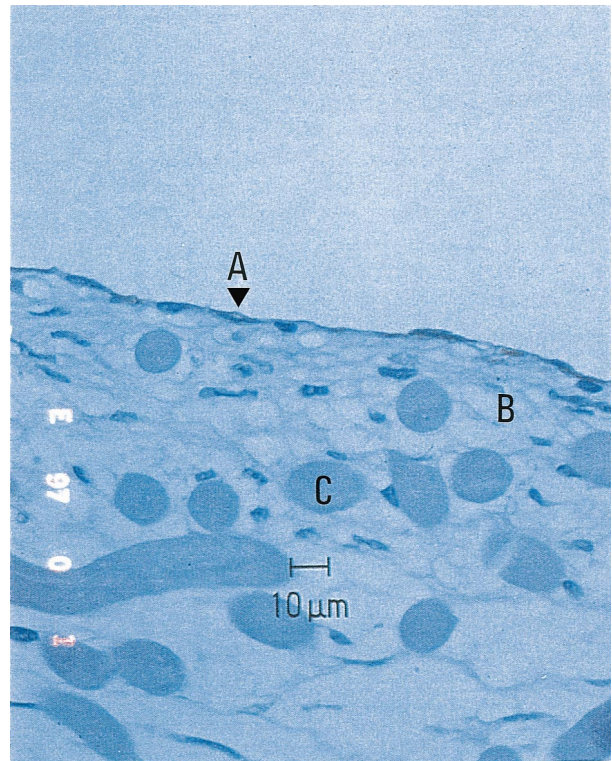


Fig. 3. Formation of endothelial monolayer. CD 34 stain demonstrates a monolayer of endothelial cells (A) on the surface of fibroblasts core (B). After 4 weeks hydrolysis of polymer fibers (C) is in process.

The production of collagen matrices was demonstrated by staining the tissue-like structure with Van Gieson. There was evidence of beginning hydrolytic degradation of the polymeric fibers. The seeded endothelial cells formed a monolayer on the human fibroblasts as demonstrated by factor VIII and CD 34 (Fig. 3). In addition, no invasive growth of endothelial cells or new formation of capillaris could be detected.

#### 3.2. Electron microscopy

The scanning electron microscopic examination 7 days before seeding with human aortic endothelial cells demonstrated attachment of fibroblasts to the polymeric fibers and the formation of cell bridges between polymeric fibers. In addition a homogeneous distribution of the seeded fibroblasts could be detected. There was the same density of fibroblasts in the upper and lower part of the PGA mesh and no gravity gradient could be detected. Some of the polymeric fibers have started hydrolytic degradation.

### 4. Discussion

In tissue engineering, the material properties of synthetic compounds are chosen to enable delivery of

dissociated cells into a host with successive formation of new functional tissue. The advantage of such tissue engineered products is the ability to grow, repair and remodel, and no requirement for anticoagulation. Furthermore, if autologous cells are used for the construction of such products, an increasing durability will be achieved with lack of risk of immunogenicity and rejection. The new field of tissue engineering is emerging experimentally and tissue substitutes for liver, cartilage, bone, trachea, intestine and urologic tissue are created [7–10]. The first tissue engineered products in clinical use are skin replacements. In the field of cardiac surgery tissue engineering is not unknown. Marelli et al. demonstrated that skeletal muscle satellite cells can be harvested, grown in vitro, and autotransplanted into damaged heart muscle [11–13]. Preliminary results showed that muscle formation occurred at 8 weeks, but not at 14 weeks after transplantation [12]. Apart from that, the group of Vacanti and Mayer at Children's Hospital in Boston has become a leading group in cardiovascular tissue engineering [4–6]. The goal of their research is to create an autologous, tissue engineered aortic valve with the ability to grow, repair and remodel. They created an animal model in which a pulmonary valve leaflet was successfully replaced by an autologous tissue engineered valve leaflet. Furthermore at our institution autologous capillary endothelial cells have been harvested, grown in vitro and seeded onto artificial vascular grafts in order to promote hemocompatibility [14–17]. In our experiences, endothelial cell transplantation has failed to make a significant transition from the animal laboratory to the clinical area. We believe nowadays that human endothelial cells need a living compound for normal growth and survival in the human blood stream. Since the seeding of human fibroblasts on biodegradable mesh is a precondition of any tissue engineered product in cardiovascular surgery we first focused upon the development and evaluation of an adequate method. For this reason we started with creating a living compound of fibroblasts followed by seeding of endothelial cells. Only endothelial cells of the human ascending aorta were used suggesting that human capillary endothelial cells thus be angiogenic and might form new capillaries. For the development of a living compound we choose preputial fibroblasts from newborns. Since in contrast to adults this cells have a higher potential for growth and higher cell generations can be presumed [18]. The most important steps in this study are the attachment of human fibroblasts to the fibers and the formation of cell bridges and in addition the seeding of endothelial cells forming a monolayer with no evidence of invasion or formation of capillary structures into the core. The polymer templates that we utilize are both biocompatible and biodegradable; a rapid degradation is desirable in order to minimize any chronic foreign-body inflammatory response [19]. The

next step in our study will involve the sorting out of a mixed cell population obtained from human ascending aorta to pure cell populations followed by serial passaging to obtain sufficient numbers of cells for cell seeding.

Cardiovascular tissue engineering has the potential to improve our ability to treat cardiovascular disease, one of the most important single cause of mortality in the industrial world. The preliminary results of this study represent a basic step on the way to construct human cardiovascular tissue and demonstrate that the applied technique of tissue engineering might be a feasible approach.

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## Appendix A. Conference discussion

**Dr T. Ferguson** (*St. Louis, USA*): You caught me a little by surprise because the abstract does not refer specifically to using this approach on valves, which to me is the most difficult experimental environment. I guess my question would be, is this technique applica-

ble to vascular grafts and other biologic situations?

**Dr Zünd:** Yes. I do agree with you. In one of our animal studies we were just replacing a pulmonary leaflet.

**Dr Ferguson:** Well, if it works in a valve, it will work anywhere in the body, of that you can be sure.

**Dr C. Muneretto** (*Padua, Italy*): We have already developed a research program on tissue engineering. And in our experience we use human vessel treated to suppress host-immune response, and we use this vessel as a geometrical and mechanical support for the seeding and growth of fibroblasts and endothelial cells. We found two main problems. The first one, the endothelial growth; and the second one, membrana basalis formation.

I have two questions for you. Did you use any method to increase speed in endothelial cell growth? And second question, did you find any formation of membrana basalis in you conduit?

**Dr Zünd:** First of all, we don't use any additional factors for endothelial growth. We just are culturing them, and then, after some passages, we are seeding them on the fibroblast core. Second, we were not yet looking for the basilar membrane.

**Dr Ferguson:** Were you able to show by low microscopic scans, that total coverage of the surface is achieved?

**Dr Zünd:** Yes, sir. The fibroblasts were totally confluent and formed a new tissue.